Microbiome-Sparing Phage Endolysins to Dissolve the *Gardnerella* Biofilm and Break the Cycle of Recurrent Bacterial Vaginosis

Project Summary / Abstract

Bacterial vaginosis (BV) is the most common vaginal dysbiosis among reproductive-age women and is driven by replacement of protective *Lactobacillus* species with a polymicrobial, *Gardnerella*-anchored biofilm adherent to the vaginal epithelium. First-line antibiotics (metronidazole, clindamycin) penetrate this biofilm poorly and indiscriminately suppress commensal lactobacilli; consequently, recurrence within 3–12 months exceeds 50%. Phage-derived endolysins (peptidoglycan hydrolases) are mechanistically matched to this problem: an engineered, domain-optimized endolysin can be made *Gardnerella*-selective, and—because it attacks the peptidoglycan scaffold from the outside—can enzymatically dissolve the established biofilm at low microgram concentrations where antibiotics fail. The lead molecule in this class, PM-477 (and the single-amino-acid variant BNT331), kills tested *Gardnerella* species at low MIC90, eradicates biofilms at single-digit µg/mL, and selects resistance far more slowly than metronidazole (Landlinger et al., 2022). In an ex vivo study of vaginal samples from 49 women with BV, the endolysin reduced viable *Gardnerella* by ≥94% at 20–50 µg/mL over 19 h while *L. crispatus* proliferated where present; notably, the transitional species *L. iners* was concurrently reduced (~92%), indicating selective steering toward an *L. crispatus*–dominant state rather than indiscriminate sparing (Tisakova et al., 2025). A completed Phase I trial (NCT06469164) evaluated a BNT331 vaginal insert. This R01 will establish the U.S. translational foundation for the approach by (1) mapping endolysin susceptibility across the strain/clade diversity of *Gardnerella* in a U.S. recurrent-BV population, (2) defining the mechanistic basis of microbiome-steering biofilm clearance and resistance resilience, and (3) testing whether endolysin debulking plus an *L. crispatus* probiotic enables durable, *L. crispatus*–dominant re-colonization. The work is squarely responsive to NIAID's mission in antibiotic alternatives and microbiome-targeted anti-infectives, with secondary relevance to reproductive-health and preterm-birth priorities.

Specific Aims

Recurrent BV is a high-burden, poorly solved infectious-disease problem: antibiotics fail on two

fronts—biofilm recalcitrance and collateral destruction of the protective lactobacillus community—and recurrence exceeds 50% within a year. *Gardnerella*-selective phage endolysins offer a mechanistically distinct solution by lysing *Gardnerella* and dissolving its biofilm peptidoglycan while preserving the most protective commensal, *L. crispatus*. Building only on published preclinical, ex vivo, and Phase I evidence for the engineered *Gardnerella* endolysins PM-477/BNT331 and on academic endolysin-discovery work, we will define the determinants of efficacy, selectivity, and durability needed to position this modality for U.S. clinical development in recurrent BV.

Aim 1. Define the breadth of endolysin susceptibility across the strain and clade diversity of *Gardnerella* in U.S. recurrent-BV patients. Because *Gardnerella*'s deep strain/clade diversity narrows whole-phage host range, we will assemble a U.S. clinical-isolate panel and measure planktonic susceptibility (MIC, MIC90) and minimum biofilm eradication concentrations (MBEC) for a *Gardnerella*-selective endolysin, benchmarked head-to-head against metronidazole and clindamycin. *Hypothesis:* cell-wall-binding-domain (CBD) recognition confers uniform coverage across U.S. clades. *Go/no-go:* ≥80% of clade-representative isolates inhibited at ≤4× the published PM-477 MIC90 [ILLUSTRATIVE].

Aim 2. Establish the mechanistic basis of microbiome-steering biofilm dissolution and resistance resilience. Using mixed *Gardnerella–Lactobacillus* biofilms and serial-passage selection, we will quantify endolysin-mediated peptidoglycan degradation and biofilm disruption, confirm *L. crispatus* sparing, characterize the differential effect on *L. iners*, and test whether resistance remains marginal over extended passaging, as reported for PM-477. *Hypothesis:* CBD specificity drives selective lysis of *Gardnerella* and *L. iners* while sparing *L. crispatus*; a conserved structural target limits resistance.

Aim 3. Test endolysin debulking plus *L. crispatus* probiotic re-colonization in ex vivo / preclinical models of the recurrence cycle. In ex vivo vaginal-sample and complementary preclinical models, we will determine whether endolysin debulking creates a niche permitting durable *L. crispatus* engraftment and suppressed *Gardnerella* rebound, modeling a microbiome "reset" rather than transient symptom clearance.

Impact. Success would provide the U.S. mechanistic and translational foundation for a non-antibiotic, biofilm-dissolving, *L. crispatus*–preserving treatment for recurrent BV, with downstream relevance to reproductive-health outcomes.

Significance

BV is the most common cause of vaginal dysbiosis in reproductive-age women, and its core pathology is not simple overgrowth but a structured, *Gardnerella*-anchored polymicrobial biofilm adherent to the vaginal epithelium that displaces protective lactobacilli. This biofilm is the proximate

reason standard care fails to deliver durable cures. Metronidazole and clindamycin relieve acute symptoms but penetrate the biofilm poorly and simultaneously suppress commensal *Lactobacillus* species, leaving the epithelial biofilm scaffold intact and the niche depleted of the organisms that normally exclude *Gardnerella*. The clinical consequence is a revolving door of recurrence exceeding 50% within 3–12 months. Recurrent BV is therefore an unsolved infectious-disease problem squarely within NIAID's remit on antibiotic alternatives and microbiome-targeted anti-infectives, and—through its established links to reproductive health and preterm birth—an area of secondary NICHD interest.

Endolysins address the two specific failure points of antibiotics. First, they hydrolyze peptidoglycan from outside the cell, acting directly on the structural matrix that holds the *Gardnerella* biofilm together and achieving biofilm eradication at single-digit µg/mL where metronidazole and clindamycin underperform (Landlinger et al., 2022). Second, an engineered CBD confers *Gardnerella* selectivity at low MIC90 (Landlinger et al., 2022). Critically, the selectivity profile observed in clinical material is more nuanced—and more favorable—than simple "spare-all-lactobacilli" logic: in vaginal samples from 49 women with BV, the endolysin reduced viable *Gardnerella* by ≥94% at 20–50 µg/mL over 19 h while *L. crispatus* proliferated where present, yet *L. iners* was concurrently reduced (~92%) (Tisakova et al., 2025). Because *L. crispatus* dominance (community state type I) is the most stable, protective vaginal state whereas *L. iners* (CST III) is transitional and frequently precedes relapse, an agent that clears *Gardnerella*, depletes *L. iners*, and spares *L. crispatus* may actively steer the community toward durable health rather than merely suppressing symptoms. Because the target is a conserved structural wall rather than a mutable metabolic pathway, resistance arose only marginally over extended passaging, in contrast to rapid high-level metronidazole resistance (Landlinger et al., 2022). By rigorously testing these properties against U.S. clinical diversity and linking biofilm debulking to *L. crispatus* re-colonization, this project would move the field beyond proof-of-concept toward a durable, microbiome-steering cure.

Innovation

This proposal is innovative in mechanism, target, and therapeutic strategy. (1) **Mechanism:** rather than a metabolic antibiotic or a host-range-limited whole phage, it centers on a phage-derived endolysin that enzymatically dissolves the biofilm peptidoglycan scaffold—turning the biofilm from an obstacle into the drug's substrate. (2) **Selectivity as a design principle:** CBD engineering removes *Gardnerella* while preserving *L. crispatus*, inverting the collateral-damage logic of antibiotics. (3) **Microbiome steering, not just sparing:** we reframe the observed concurrent depletion of the transitional species *L. iners* alongside *L. crispatus* sparing (Tisakova et al., 2025) as directional steering toward the protective CST-I state—an underused conceptual lever in BV therapeutics. (4) **Resistance resilience:** targeting a conserved structural wall yields only marginal MIC shifts over

extended passaging, offering durability where metronidazole resistance emerges rapidly. (5) **Curative strategy:** pairing endolysin debulking with an *L. crispatus* probiotic reframes treatment as a one-time microbiome "reset" enabling re-colonization. (6) **Translational positioning:** the project builds directly on a completed Phase I program (NCT06469164) and published ex vivo pharmacodynamics, applying that momentum to U.S. clinical-isolate diversity and recurrence biology—an evidence-grounded, de-risked path.

Approach

Overarching rigor. All quantitative assays will use ≥3 biological replicates and prespecified statistical tests with correction for multiple comparisons; isolate panels will be powered to detect clade-level differences in susceptibility [ILLUSTRATIVE]. Because BV is a condition of the female reproductive tract, all human-derived specimens are necessarily female; this is a disease-defined population rather than an unjustified exclusion, and we will analyze results across relevant demographic and community-state-type strata. Endolysin reagents will be the published PM-477 / BNT331 sequences or functionally equivalent CBD-matched constructs; identity and activity will be confirmed against published activity ranges as assay-qualification anchors.

Aim 1 — Susceptibility and biofilm eradication across U.S. *Gardnerella* clade diversity

Rationale. *Gardnerella*'s deep strain/clade diversity is the principal reason whole-phage host range is narrow and motivates protein-based, genus-selective approaches. Genus-level activity has been shown for PM-477 against tested *Gardnerella* species (Landlinger et al., 2022), but coverage across a contemporary U.S. recurrent-BV isolate collection must be established directly.

Experimental design. Under IRB approval (clinical core), we will assemble a panel of *Gardnerella* clinical isolates from U.S. recurrent-BV patients, assign species/clade by validated genomic typing, and determine planktonic susceptibility (MIC, MIC90) for a *Gardnerella*-selective endolysin. We will grow established single-strain biofilms and measure MBEC, benchmarked head-to-head against metronidazole and clindamycin, anchoring assays to published activity ranges (low-µg/mL MIC90; single-digit-µg/mL biofilm eradication; Landlinger et al., 2022). Additional anti-*Gardnerella* endolysins reported by academic groups (Arroyo-Moreno et al., 2022) provide candidate backups and comparators.

Expected outcomes. A quantitative susceptibility/MBEC map across U.S. clades, defining the breadth (and any bounds) of genus-level coverage and quantifying superiority over antibiotics in biofilm assays.

Go/no-go & pitfalls. *Success:* ≥80% of clade-representative isolates inhibited at ≤4× the published MIC90 [ILLUSTRATIVE]. If certain clades prove less susceptible, we will test whether CBD-recognition gaps explain it and evaluate alternative or pooled endolysins from the academic pipeline (Arroyo-Moreno et al., 2022) as a cocktail to widen coverage.

Aim 2 — Mechanism of microbiome-steering biofilm dissolution and resistance resilience

Rationale. The therapeutic promise rests on two mechanistic claims: selective peptidoglycan attack that dissolves the *Gardnerella* biofilm while sparing *L. crispatus*, and slow resistance evolution against a conserved target (Landlinger et al., 2022). The ex vivo observation that *L. iners* is co-depleted while *L. crispatus* proliferates (Tisakova et al., 2025) must be dissected mechanistically, not assumed away.

Experimental design. We will construct mixed *Gardnerella–Lactobacillus* biofilms and quantify endolysin-mediated peptidoglycan degradation and biofilm disruption (structural and viability readouts), explicitly measuring effects on both *L. crispatus* and *L. iners* to map the basis of differential susceptibility (e.g., CBD–ligand and cell-wall compositional differences). In parallel, we will perform serial-passage selection over an extended passage series modeled on the published PM-477 protocol [ILLUSTRATIVE] to determine whether MIC shifts remain marginal, directly contrasting with rapidly selected metronidazole resistance.

Expected outcomes. Mechanistic confirmation that the endolysin dissolves the *Gardnerella* biofilm matrix and depletes *L. iners* while leaving *L. crispatus* viable, with a molecular account of the *L. crispatus*/*L. iners* differential, plus evidence that resistance remains marginal over extended passaging.

Go/no-go & pitfalls. If *L. crispatus* viability is unexpectedly affected, we will map the responsible binding interactions and prioritize CBD variants with the cleanest *L. crispatus*-sparing profile. If resistance emerges, we will characterize the mechanism and test endolysin combinations to suppress it.

Aim 3 — Endolysin debulking plus *L. crispatus* re-colonization in models of the recurrence cycle

Rationale. Durable cure requires re-establishing an *L. crispatus*-dominant community, not merely killing *Gardnerella*. Ex vivo, endolysin treatment reduced viable *Gardnerella* by ≥94% while *L. crispatus* proliferated and *L. iners* fell (Tisakova et al., 2025), suggesting debulking can open the niche for *L. crispatus* re-colonization.

Experimental design. Using ex vivo vaginal samples from women with BV—mirroring the design that defined the human-relevant dose (≈19 h at 20–50 µg/mL; Tisakova et al., 2025)—and complementary preclinical models, we will test whether endolysin debulking followed by *L. crispatus* probiotic supplementation yields durable *L. crispatus* engraftment and suppressed *Gardnerella* (and *L. iners*) rebound, modeling a microbiome "reset" versus transient clearance. Sequencing and timing of debulk-then-probiotic delivery will be varied systematically.

Expected outcomes. Evidence that endolysin debulking enables sustained *L. crispatus* re-colonization and reduced *Gardnerella* rebound, supporting a debulk-plus-probiotic regimen.

Go/no-go & pitfalls. Ex vivo and preclinical models incompletely capture host immunity and the menstrual cycle; we will interpret durability cautiously and triangulate across models. If probiotic engraftment is inconsistent, we will optimize timing/sequencing relative to debulking and consider endolysin–probiotic co-formulation strategies.

Timeline

- **Year 1 [ILLUSTRATIVE]:** Assemble U.S. *Gardnerella* clinical-isolate panel; clade typing; planktonic MIC/MIC90; assay qualification (Aim 1).
- **Years 1–2 [ILLUSTRATIVE]:** MBEC mapping vs. antibiotics (Aim 1); initiate mixed-biofilm mechanism and serial-passage studies (Aim 2).
- **Years 2–3 [ILLUSTRATIVE]:** Complete mechanism and resistance work, including *L. crispatus*/*L. iners* differential (Aim 2); begin ex vivo debulking + probiotic studies (Aim 3).
- **Years 3–4 [ILLUSTRATIVE]:** Preclinical re-colonization models; integration and durability analyses (Aim 3).
- **Year 5 [ILLUSTRATIVE]:** Cross-aim synthesis; regulatory/translational data packaging for next-phase U.S. clinical planning.

Budget Justification (modular R01-style sketch)

This is a modular R01 request at $250,000 direct costs per year [ILLUSTRATIVE] for 5 years [ILLUSTRATIVE]. **Personnel:** PI (microbiology/infectious disease; ~2.4 calendar months [ILLUSTRATIVE]); Co-I clinical (BV/reproductive infectious disease) for isolate/sample collection and human-subjects oversight (~1.2 months [ILLUSTRATIVE]); Co-I microbiome/biofilm scientist; two research technicians/postdocs [ILLUSTRATIVE] for susceptibility, biofilm, mechanism, and ex vivo work. **Supplies:** anaerobic culture and biofilm consumables, endolysin protein, *Lactobacillus* (incl. *L. crispatus*) probiotic strains, molecular/clade typing, and peptidoglycan-degradation assays [ILLUSTRATIVE]. **Other:** clinical-core costs for IRB-approved sample acquisition, biostatistics, and

dissemination [ILLUSTRATIVE]. No major equipment is requested [ILLUSTRATIVE]. An alternate **R43/R44 SBIR** pathway would suit an industry-partnered product-development scope, and **NICHD** co-funding may be sought given the preterm-birth/reproductive-health linkage.

Vertebrate Animals

If preclinical in vivo re-colonization models in Aim 3 require vertebrate animals, a complete Vertebrate Animals Section with IACUC approval, justification of species and numbers [ILLUSTRATIVE], minimization of pain/distress, and humane endpoints will be provided; non-animal ex vivo vaginal-sample models will be prioritized to reduce animal use. If the final design is limited to ex vivo and in vitro work: Not applicable.

Human Subjects / Clinical Trial

This project is not itself an interventional clinical trial; human involvement is limited to IRB-approved collection of clinical *Gardnerella* isolates and ex vivo vaginal samples from women with BV (Aims 1 and 3), consistent with the published ex vivo study of samples from 49 women (Tisakova et al., 2025). Because BV is a disease of the female reproductive tract, the study population is necessarily female; results will be analyzed across relevant demographic and community-state-type strata. All sample acquisition will proceed under institutional IRB oversight with informed consent and a data-safety monitoring plan appropriate to minimal-risk specimen research. Should any in-human administration of investigational endolysin/phage be pursued in a future phase, we note the FDA emergency/expanded-access IND route available for investigational phage therapeutics, alongside standard IND requirements; the registered Phase I program for BNT331 (NCT06469164) provides regulatory precedent for the vaginal-insert route.

Team & Environment

- **Principal Investigator — [Name, Institution]:** infectious-disease microbiologist with anaerobic culture and endolysin/biofilm expertise.
- **Co-Investigator (Clinical) — [Name, Institution]:** OB/GYN or reproductive infectious-disease physician leading IRB-approved isolate/sample acquisition from U.S. recurrent-BV patients.
- **Co-Investigator (Microbiome/Biofilm) — [Name, Institution]:** expertise in *Gardnerella* biofilm and endolysin testing.
- **Biostatistician — [Name, Institution].**
- **Consultants/Collaborators — [Names]:** endolysin engineering and regulatory/translational

advisors.

- **Environment:** institutional anaerobic microbiology and biofilm facilities, molecular/clade typing, an IRB-approved clinical core for sample collection, and biostatistics support [ILLUSTRATIVE], sufficient to execute all aims.

References

1. Tisakova LP, Schwebs T, Berdaguer R, von Freyberg M, Foessleitner P, Kieninger AK, Poljak A, Corsini L, Farr A. Endolysin selectively kills *Gardnerella* ex vivo in vaginal samples from women with bacterial vaginosis. *npj Biofilms and Microbiomes.* 2025;11(1):161. https://pubmed.ncbi.nlm.nih.gov/40796753/
2. Landlinger C, Oberbauer V, Podpera Tisakova L, Schwebs T, Berdaguer R, Van Simaey L, Vaneechoutte M, Corsini L. Preclinical Data on the *Gardnerella*-Specific Endolysin PM-477 Indicate Its Potential to Improve the Treatment of Bacterial Vaginosis through Enhanced Biofilm Removal and Avoidance of Resistance. *Antimicrobial Agents and Chemotherapy.* 2022;66(5):e02319-21. https://doi.org/10.1128/aac.02319-21
3. Arroyo-Moreno S, Cummings M, Corcoran DB, Coffey A, McCarthy RR. Identification and characterization of novel endolysins targeting *Gardnerella vaginalis* biofilms to treat bacterial vaginosis. *npj Biofilms and Microbiomes.* 2022;8(1):29. https://doi.org/10.1038/s41522-022-00285-0
4. BioNTech SE. A Phase I Randomized, Double-blind, Placebo-controlled, Safety, Tolerability, and Pharmacokinetic Trial of BNT331 Administered in Single Ascending Doses in Healthy Women and Multiple Ascending Doses in Women Diagnosed With Bacterial Vaginosis (BNT331-01). ClinicalTrials.gov identifier NCT06469164; started 2024, status Completed. https://clinicaltrials.gov/study/NCT06469164